REMARKS

Reconsideration is requested.

Claims 12, 15, 16, 46, 49 and 50 have been additionally canceled above, without prejudice.

Claims 1, 4, 10, 13, 14, 40, 43, 44, 47 and 48 are pending.

The separate Section 112, first paragraph "enablement", rejections of claims 1, 4, 10, 13, 40, 43, 44 and 47; and claims 12, 14-16, 46 and 48-50, are traversed. Reconsideration and withdrawal of the rejections are requested in view of the above and the following comments.

The applicants understand the Examiner to assert that the specification enables one of ordinary skill in the art to make and use plants and plant cells transgenic for nucleic acid sequences encoding Arabidopsis E2Fa and GPa transcription factors. The applicants respectively submit that one of ordinary skill in the art will be able to make and use the claimed invention without requiring undue experimentation.

The claims are based on introducing and expressing a nucleic acid which is at least 95% identical to SEQ ID NO: 1835 or to a sequence encoding SEQ ID NO: 1836. One of ordinary skill in the art will be able to introduce and to express a specific nucleic acid in a plant cell without undue experimentation. The specification further describes such methods in, for example, ¶ [0021] of the U.S. Patent Office published version of the specification (i.e., US 2006-0021088).

Moreover, the following is a description of experiments carried out by the applicants or under the control or direction of the applicants, wherein a nucleic acid

having a sequence as shown in SEQ ID No. 1835 was introduced into plants and expressed under a) the control of a constitutive promoter (GOS2 promoter) and b) under the control of a seed-specific promoter (prolamin-promoter). It was shown that the resulting transgenic plant had increased yield, regardless whether said nucleic acid was under the control of the constitutive promoter or the seed-specific promoter (see table I for the constitutive promoter; see table II for the seed-specific promoter). Thus, expression of nucleic acid having a sequence as shown in SEQ ID No 1835 as well as a polypeptide encoded by said polynucleotide results in increased yield and biomass.

Example A: SEQ ID NO: 1835 under the control of the constitutive promoter GOS2

A DNA fragment encoding the 2XC2H2 protein represented in the application by SEQ ID NO: 1836 was isolated from an *Arabidopsis thaliana* seedling cDNA library (Invitrogen, Paisley, UK). PCR was performed using Hifi Taq DNA polymerase in standard conditions, using 200 ng of template in a 50 µl PCR mix. The primers used for the PCR amplification (and which include the AttB sites for Gateway recombination) were as follows:

Forward primer: ggggacaagtttgtacaaaaaagcaggcttaaacaatggccctcgaagcg

Reverse primer: ggggaccactttgtacaagaaagctgggttcgagtattagatttttaaagataaatc

The amplified PCR fragment was purified also using standard methods. The first step of the Gateway procedure, the BP reaction, was then performed, during which the PCR fragment recombined *in vivo* with the pDONR201 plasmid to produce, according to

the Gateway terminology, an "entry clone". Plasmid pDONR201 was purchased from Invitrogen, as part of the Gateway® technology.

The entry clone comprising SEQ ID NO: 1835 was then used in an LR reaction with a destination vector used for *Oryza sativa* transformation. This vector contained as functional elements within the T-DNA borders: a plant selectable marker; a screenable marker expression cassette; and a Gateway cassette intended for LR *in vivo* recombination with the nucleic acid sequence of interest already cloned in the entry clone. A rice GOS2 promoter for constitutive specific expression was located upstream of this Gateway cassette. After the LR recombination step, the resulting expression vector (pGOS2::SEQID1835) was transformed into *Agrobacterium* strain LBA4044 according to methods well known in the art.

Rice transformation and phenotypic evaluation of the plants was as described in Example 12.

The results are shown in Table I below.

Table I: Results of phenotypic characterization of rice plants transformed with pGOS2::SEQID1835. Overall percentage of increase is given for biomass (above ground area), total seed number and for flowers per panicle for T1 and T2 plants.

	T1 generation		T2 generation	
Parameter	% increase	p-value	% increase	p-value
Above ground area	10.5	0.002	9.5	0.000
Total number of seeds	9.7	0.031	11.9	0.001
Flowers per panicle	7.8	0.000	8.0	0.001

In addition, an increase was observed for seed fill rate (3 positive lines out of 4 in T2, overall increase of 30.1% with a p-value of 0.000), for harvest index (3 positive lines

out of 4 in T2, overall increase of 35.0% with a p-value of 0.000) and for thousand kernel weight (2 positive lines out of 4 in T2, overall increase of 1.9% with a p-value of 0.005).

Example B: SEQ ID NO: 1835 under the control of the seed-specific promoter prolamin

Cloning of SEQ ID NO: 1835 was as described above. The entry clone was subsequently used in an LR reaction with a destination comprising the seed-specific prolamin promoter (mentioned in Example 12). Plant transformation and phenotypic analysis were as described above and as described in Example 12.

Table II: Results of phenotypic characterization of rice plants transformed with pPROLAMIN::SEQID1835.

	T1 generation		T2 generation	
Parameter	% increase	p-value	% increase	p-value
Early vigour	25.7	0.001	10.4	0.021
Total seed weight	17.7	0.001	8.3	0.007
Total number of seeds	11.0	0.003	5.1	0.049
Seed fill rate	7.4	0.014	13.8	0.123
Harvest index	14.3	0.000	5.1	0.040
Number of filled seeds	19.5	0.000	7.2	0.018

In addition, an increase was observed for thousand kernel weight (2 positive lines out of 4 in T2, overall increase of 3.2% with a p-value of 0.052).

The applicants believe the above demonstrates that the claims are supported by an enabling disclosure.

The applicants further understand the Examiner to believe that plants transgenic for a sequence that is natively expressed are not always predictably obtainable, since native and recombinant expression conditions may not be equivalent. Moreover, the Examiner is further understood to believe that plants having modified levels or activities of a protein are not always predictably obtainable, since conditions that modify gene expression would allegedly not always modify the level or activity of the protein encoded by the gene. The Examiner refers to Sakamoto et al., and Temple et al. in this regard.

According to the Examiner, Temple would teach the introduction of antisense constructs aimed specifically at two distinct classes of gluatmine synthetase-1 genes into alfalfa. While the document Temple would show a 80% reduction of the transcript level of the corresponding genes, no reduction in glutamine synthetase activity and of the glutamine synthetase-1 polypeptide level was observed.

The applicants believe however that the Examiner's reliance on Temple is misplaced and that one of ordinary skill in the art can not compare the results obtained by Temple with the subject matter of the claims. Specifically, the claims relate to the expression of a nucleotide sequence as shown in SEQ ID NO:1835, rather than to the down-regulation of said sequence. The applicants submit that expression of a nucleic acid and down-regulation of a nucleic acid, however, are two distinct approaches. Whereas the expression of nucleic acid results in increased expression of a nucleic acid that is introduced into a plant cell, the down-regulation aims to reduce the level of a transcript which is present in a cell. Moreover, the applicants submit that Temple was published in 1998 and, thus, at a time at which the entire sequence of plant genomes

was not known (the first plant genome has been published in 2000 for Arabidopsis). Thus, the results reported in Temple are not believed to be instructive of the level of skill in the art at the time of the present application.

The applicants further understand the Examiner to believe that Sakamoto would teach that the native expression of AZF2 (SEQ ID NO:1835) could be induced by various stresses. However, Arabidopsis plants transgenic for the AZF2 coding sequence under the control of the CaMV 35S promoter over-expressing AZF2 could not be obtained.

The applicants have shown that a nucleic acid having a sequence as shown in SEQ ID NO:1835 can be expressed in a plant cell (e.g. under the control of the GOS2 promoter or the prolamin-promoter) resulting in increased yield.

Reconsideration and withdrawal of the Section 112, first paragraph "enablement", rejections are requested.

The Section 101 rejection of claims 15 and 49 is moot in view of the above amendments.

To the extent not obviated or made moot by the above amendments, the Section 102 rejection of claims 12, 14-16, 46 and 48-50 over De Veylder (EMBO J. 2002 March 15;21(6):1360-8), is traversed. Reconsideration and withdrawal of the rejection are requested in view of the above and the following.

The applicants submit that de Veylder et al. does not disclose introducing and expressing a polynucleotide having a sequence of the claims into a plant or a plant cell, as claimed. Moreover, a polynucleotide as shown in SEQ ID NO:1835 is not over-

expressed in plants transgenic for Arabidopsis E2Fa and DPa transcription factors. Rather, the applicants submit that said polynucleotide is repressed in E2Fa/ DPa plants (see table 5 of the specification). Accordingly, the claims are submitted to be patentable over the cited art and withdrawal of the Section 102 rejection is requested.

The claims are submitted to be in condition for allowance and a Notice to that effect is requested. The Examiner is requested to contact the undersigned, preferably by telephone, in the event anything further is required.

Respectfully submitted,

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